We prepared the kinesin motor domain (K355) mutant that has a single cysteine at neck linker region and His-tag at C-terminal. Subsequently the kinesin mutant was dimerized with photochromic bifunctional cross-linker, azobenzene dimaleimide (ABDM). And the photo-reversible regulation of the ATPase and motor activities of the kinesin dimer cross-linked with ABDM was studied.
We also tried to develop photo-responsive vesicle composed of photochromic molecules as a cargo for the photo-controlled kinesin. Diacyl glycerol was coupled with carboxypropyl-spiropyran to be phospholipid analogue using carbonyldiimidazole condensation reagent. The spiropyran moiety performs photo-reversible isomerization between hydrophobic spiro form and merocyanine zwitterion form upon visible light and ultraviolet light, respectively. Therefore, it is expected that the merocyanine form of the mimic phospholipid results in formation liposome like vesicle. The photo-reversible formation of the vesicle was studied using water-soluble fluorescent probe.

680-Pos Board B460
Study of Phospho-Regulation of a Mitotic Kinesin using a Directed Evolution Approach
Alina Goldstein[1,2], Nurit Siegler[1,2], Liam Holt[3], Leah Gheber[1,2].
[1]Department of Chemistry, Ben Gurion University of the Negev, Beer Sheva, Israel, [2]Ilse Katz Institute for Nanoscale Science and Technology, Ben Gurion University of the Negev, Beer Sheva, Israel, [3]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA.
The *S. cerevisiae* Cin8 belongs to the kinsesin-5 sub-family of mitotic motor proteins. During mitosis, Cin8 orchestrates the mitotic spindle assembly and its elongation. Recent work from our laboratory indicated that phosphorylation of Cin8 by Cdk1 governs its localization to the mitotic spindle during mitosis. Here we tested the rigidity of phosphorylation sites in Cin8, and examined whether phosphorylation at newly created Cdk1 sites can mimic the known phospho-regulation or create new regulation. For this purpose, we generated phosphorylation-deficient mutant of Cin8 and introduced new Cdk1 sites by single amino acid replacement. This resulted in thirty-one novel Cdk1 phosphorylation sites. In part of the sites, partial and full Cdk1 consensus sites were created. Next we analyzed Cin8 localization to the spindle during anaphase. We found that only one novel Cdk1 phosphorylation site at position 276 is able to restore the original phospho-regulation of Cin8, and is located in high proximity to a native Cdk1 phosphorylation site (S277). Although several sites were created nearby, only this site exhibits localization pattern which is similar to WT-Cin8. This result suggests that phospho-regulation of Cin8 by Cdk1 at this region is rigid and highly dependent on the structural context. Several additional novel Cdk1 mutants exhibited new phenotypes, suggesting that there are regions in Cin8 where phsopho-regulation by Cdk1 is more flexible. These results imply that phospho-regulation of Cin8 is more elusive than previously anticipated and further study of its mechanism is required.

681-Pos Board B461
Transport by a Kinesin in the Presence of Magnetic Nanoparticles
Ehsan Mirzakhalili, Eleni Gourgou, Bogdan Epureanu.
Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA.
Superparamagnetic nanoparticles are used to influence the medium in which kinesin nanotransport occurs. Simulation results show that nanoparticles form chain-like structures aligned with the direction of applied external magnetic field. The strength of the links in these chains depends on the properties of the particles and on the intensity of the applied magnetic field. Therefore, altering the magnetic field can be used to dynamically control the loads kinesins have to overcome - analogous to modifying properties of the medium in which the transport takes place.
The components of the motor protein, namely its two heads, two neck linkers and a neck and a cargo linker, are considered to be linear elastic elements. The chemical reaction of ATP/ADP and the heads is modeled using Michaelis-Menten kinetics and the Arrhenius equation. The overall model is shown to successfully capture the hand-over-hand motion of kinesin. By simulating the transport of a cargo by kinesin through obstacles created by the magnetic nanoparticles, it is shown that the resisting force created by chains of magnetic nanoparticles affects the speed of kinesin transport.
However, characterizing the motion of a kinesin in the presence of many magnetic nanoparticles requires stochastic simulations at a variety of conditions. The required computational time is prohibitive. Hence, a generalized model is developed to estimate the force on the cargo without solving the full-order system dynamics every time. Finally, the motion of cargo under varying magnetic fields is studied. These results can be used to detect possible deficiencies in kinesin - microtubule interactions.

682-Pos Board B462
Kinetic Characterization of Rice Plant Specific Kinesin E11 using Fluorescent ATP Analogue
Hironobu Taniguchi[1], Nozomi Umezu[2], Shinsaku Maruta[1,2].
[1]Div.Bioinfo.,Grad. Sch. Eng., Soka Univ, Tokyo, Japan, [2]Dept. Bioinfo., Fac. Eng., Soka Univ, Tokyo, Japan.
Kinesin is an ATP-driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. Kinesin species derived from vertebrates have been well characterized. In contrast, plant specific kinesin have yet to be adequately characterized. We have previously demonstrated that some kinesins derived from *rice* plant have unique biochemical characteristic properties and structures.
In this study, we characterized *rice* plant specific kinesin E11 that belongs to the plant specific At1 subfamily in kinesin-7 family. E11 motor domain was expressed by *E. coli* expression system and purified with Co-chelate column in order to characterize biochemical and ATPase kinetic properties. The fluorescent ATP analogues, Mant-ATP was employed for the kinetic characterization. We have successfully observed significant FRET between Mant-ATP and intrinsic tryptophan (Trp23) residue in E11. The kinetic parameters of initial binding of Mant-ATP to E11 and release of Mant-ADP from E11 were analyzed by monitoring the FRET using stopped flow apparatus and compared with other *rice* kinesins and conventional kinesin. The results revealed that the initial binding of ATP to E11 and release of ADP are slower than those of other *rice* plant specific kinesin.

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Photo-Control of Mitotic Kinesin Eg5 using Thiol Group Reactive Fulgimide Derivative
Yuki Tamura[1], Dong Gyu Cho[2], Tae Joon Jeon[3], Shinsaku Maruta[1,4].
[1]Div.Bioinfo.,Grad.Sch.Eng., Soka University, Tokyo, Japan, [2]Chemistry, Inha University, Incheon, Korea, Republic of, [3]Biological Engineering, Inha University, Incheon, Korea, Republic of, [4]Dept.Bioinfo.,Fac.Eng., Soka University, Tokyo, Japan.
It is believed that the loop L5 of kinesin is important region for motor function. Interestingly mitotic kinesin Eg5 has a several times longer L5 in comparison with other kinesins. It has been demonstrated that the L5 of Eg5 performed as a stabilizer for the Eg5-specific inhibitors (STLC, monastrol) complexes. Aim of our study is to control the function of Eg5 photo-reversibly using photochromic molecules incorporated into L5. Previously, we have prepared Eg5 mutants (E116C,E118C,T125C,W127C,D130C) which have a single cysteine residue in L5 in order to incorporate photochromic molecules. We also synthesized thiol reactive photochromic molecules 4-phenylazomaleinanil(PAM) and Iodoacetyl-spiropyran (IASP). PAM and IASP were incorporated into the mutants stoichiometrically. Some of the Eg5 mutants modified with PAM and IASP showed reversible alteration of ATPase activity upon ultraviolet (UV) and visible (VIS) light irradiations. In this study, we synthesized a novel thiol reactive photochromic molecules monoiodoacetylfluglide(IAFG). Fulgimide performs photoreversible isomerzation between non-polar opened-ring form and polar closed-ring form upon visible light and ultraviolet light. IAFG was incorporated into Eg5 mutant W127C stoichiometrically. Althought the modified Eg 5 mutant W127C-IAFG showed slightly decreased ATPase activity, the ATPase activity showed photoreversible alteration upon UV and visible light irradiations. Alteration in the ATPase activity of W127C-IAFG in the presence of STLC upon UV and VIS light irradiations was also examined.

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Photo-Regulation of Kinesin Intramolecularly Crosslinked by Bifunctional Azobenzne Derivative at the Coiled-Coil Stalk Region
Haruka Fujio[1], Kazunori Kondo[2], Shinsaku Maruta[1,2].
[1]Bioinformatics, Soka University, Tokyo, Japan, [2]Bioinfo.,fac.eng, Soka University, Tokyo, Japan.
Kinesin is an ATP driven dimeric motor protein carries cellular cargoes along microtubules. The stalk region of kinesin is responsible for dimerization with coiled-coil interaction. Formation of dimer is essential for kinesin to perform processive movement along the microtubules. Aim of this study is to control dimerization of kinesin by the reversible conformational change at the coild-coil stalk region using photochromic molecule resulting in photo-reversible regulation of motility. Azobenzene-dimaleimide (ABDM) is a bifunctional SH reactive photochromic crosslinker and its crosslinking span is altered by cis-trans photo-isomerization of azobenznene moiety upon ultraviolet and visible light irradiations. We have previously demonstrated that the two reactive cysteine residues SH1(707) and SH2(697) in α-helix of myosin which region is believed to have a energy transducing role, were cross-linked by

ABDM. And cis-trans isomerization of the crosslinked ABDM induced disordered α-helix in the SH1-SH2 region resulting in global conformational change of myosin head.
In this study, we prepared kinesin mutant K380 cys light which has truncated minimum stalk to form dimer. Subsequently, Lys360 and Ala361 in the coiled-coli region of the mutant K380 were substituted by cysteine. The kinesin mutant K380(K350C,A361C) was modified with ABDM. The intermolecular and intramolecular crosslinking were analyzed SDS-PAGE. The optimal condition to crosslinking was 10 times excess molar ABDM for kinesin mutant and modification for 160 min at room temp. The conformational change of the ABDM crosslinked kinesin induced photoisomerization was studied with CD spectroscopy. And the photo-reversible dimerization of ABDM crosslinked kinesin was examined.

685-Pos Board B465
Investigating Cin8 Bi-Directionality as a Molecular Force Sensor
Todd Fallesen, Thomas Surrey.
Microtubule Cytoskeleton, Cancer Research UK, London, United Kingdom.
Molecular motors in eukaryotic cells are essential for many cellular functions. A diverse population of motors operate upon filaments inside the cell. The kinesin family of motors operates on microtubule (MT) filaments in tasks such as vesicle and organelle transport, mitotic spindle assembly, and chromosome separation in mitosis. A subset of kinesin motors, the kinesin-5 family, can cross-link microtubules. These cross-linking motors organize microtubules in space, which is essential for mitotic spindle assembly. In vitro experiments have demonstrated that the kinesin-5 motor Cin8, from S. cerevisiae, can switch its direction of travel on a MT. This is a remarkable property not observed for any other motor before. It has been observed through fluorescence microscopy that a single Cin8 motor on a MT moves in an ATP dependent (-) end directed manner, yet when multiple Cin8 motors crosslink two anti-parallel MTs they switch direction. This novel collective effect, essentially results from Cin8 operating not only as a force generator, but also as a novel force sensor. Using a novel binding configuration and optical force spectroscopy, we investigated the biophysical properties of Cin8 motion on microtubules as compared to the well characterized behavior of the Kinesin-1 motor. We test whether a force opposing the motion of Cin8 motors, in gliding assays, can change the direction that the Cin8 motors are collectively moving. Further, using single molecule bead assays, we investigate the behavior of single motors close to their stall force.

686-Pos Board B466
Structural-Kinetics of the Switch-1 Loop and Neck-Linker Elements Explains the Distinct Molecular Physiologies of Kinesin-1 and Kinesin-5
Joseph M. Muretta[1], Jennifer Major[2], David D. Thomas[1], Rosenfeld S. Steven[2].
[1]University of Minnesota, Minneapolis, MN, USA, [2]Cleveland Clinic, Cleveland, OH, USA.
Microtubule motors control a diverse collection of physiologies, including cell division, organelle traffic, and microtubule dynamics. Kinesins use ATP hydrolysis to power a chemical cycle that performs mechanical work. Recent, high-resolution crystallographic and cryo-EM reconstructions, single molecule mechanics, and solution kinetics studies, led to a general mechanochemical scheme for kinesin motors (Clancy, Nature Str. Mol. Biol., 18, 1020-7, (2011)). Nucleotide binding engages key structural elements, including switch I, switch II, and the P loop. Yet, how conformational changes in these switch elements lead in turn to corresponding changes in the microtubule binding domains and the motor mechanical elements (the neck linker and cover strand) remains enigmatic. Likewise, it remains unclear how the thermodynamics and kinetics of these structural transitions differ between two kinesins with very different physiologic roles. We tested two predictions of the model proposed by Clancy et al: the neck-linker gates nucleotide binding; and, coordination between the neck-linker and switch-1 fine tunes the enzymology of specific kinesin motors. We have used a recently developed technique_transient time-resolved fluorescence resonance energy transfer_which enables us to detect transitions between multiple structural states, measured with time-resolved FRET during a biochemical transient. We engineered probe-pairs to report the structure, kinetics, and equilibrium constants for nucleotide-driven structural transitions in the neck-linker and switch-1. We then compared these transitions in kinesin-1 to those in Eg5 (kinesin-5). Our results show that: 1. neck-linker docking gates nucleotide binding, as predicted by Clancy et al., and in both classes of kinesins, 2. The state of the neck-linker controls the conformation of switch-1, and 3. differences in the equilibrium constants for neck-linker docking during the microtubule bound, ATP stimulated working-stroke, explain the unique force dependence of Kinesin 1 and Eg5.

687-Pos Board B467
Processivity of Kinesin-2 Results from Rear-Head Gating and Not Front-Head Gating
Geng-Yuan Chen, David Arginteanu, William O. Hancock.
Department of Bioengineering, University Park, PA, USA.
The kinesin-2 family motor KIF3A/B coordinates with dynein to bidirectionally transport intraflagellar particles, melanosomes and neuronal vesicles. Compared to kinesin-1, kinesin-2 is less processive and its processivity is more sensitive to load, suggesting that the gating mechanisms that their control processivity may differ. To understand the motor roles that front-head gating and rear-head gating, we carried out stopped flow kinetics experiments using mant nucleotides, steady state assays, and single-molecule investigations to characterize the entire kinetic cycle a functional mouse KIF3A homodimer that exhibits similar motility to full-length KIF3A/B. Upon first encounter with the microtubule lattice, the motor exchanges mADP with an on-rate of 18 μM-1 s-1 and an off-rate of 27 s-1. When AMPPNP was used to entrap the motor in a two-head bound state, exchange kinetics were unchanged, indicating that rearward strain in the two-head-bound state does not alter nucleotide binding to the front head. Similar lack of front-head gating was found with mATP and when the neck linker domain was shortened from 17 to 14 residues to enhance intramolecular strain. In contrast, microtubule pelleting and single-molecule microscopy assays found that in ADP the motor dissociates with an off-rate of 2.1 s-1 and a KD of 0.5 μM, similar to its behavior in ATP. Hence, kinesin-2 processivity results from rear-head gating and not front-head gating. Based on the kinetics measurements, finally we propose a complete model of the kinesin-2 hydrolysis cycle that accounts for all of the kinetics and motility data. This study provides the direct evidence that rear-head gating does and not front-head gating does not play a role in kinesin-2 processivity and suggests that kinesin-2 mechanochemistry is specifically adapted for bidirectional transport.

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High Speed Microscopy for Observing the Stepping Behavior of Kinesin-1 Motors at Saturating ATP
Keith J. Mickolajczyk[1], Joanna Andrecka[2], Jaime Ortega-Arroyo[2], Philipp Kukura[2], William O. Hancock[1].
[1]Biomedical Engineering, Penn State University, University Park, PA, USA, [2]Chemistry, Oxford Univerity, Oxford, United Kingdom.
Kinesin-1 is the most well studied member of the kinesin superfamily of molecular motors and is responsible for the trafficking of vesicles and organelles towards terminal branches of axons. This motor hydrolyzes ATP in a tightly coupled fashion in order to take directed 8 nm steps along a single protofilament of a microtubule with high processivity. However, the comprehensive mechanochemical cycle of kinesin-1 remains elusive, due largely to the technical limitations of ensemble experiments and fluorescence microscopy approaches at the single-molecule level. Questions remain in identifying the rate limiting step in the hydrolysis cycle as well as the time spent in the one head bound state. To probe the mechanochemical cycle, we employ a fluorescence-free imaging technique, interferometric scattering microscopy (iSCAT), to track kinesin-1 at saturating ATP in a reconstituted system with greatly improved spatiotemporal resolution. With point spread function fitting, 8 nm and 16 nm steps are measured for C-terminus and N-terminus labeled motors, respectively. Using novel differences of means and Gaussian mixture model algorithms for the identification of step and sub-step regimes, we investigate the information present in both on-axis and off-axis positional data. Overall, these results point towards new insights into mechanochemical cycle of kinesin-1. The optical system and data analysis tools used present a new platform for investigating the stepping cycles of diverse molecular motors.

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Impact of Structural and Dynamical Complexity on Kinesin Kinetics
Bruna D. Jacobson[1], Kasra Manavi[2], Susan R. Atlas[1], Lydia Tapia[2].
[1]Dept. of Physics and Astronomy, University of New Mexico, Albuquerque, NM, USA, [2]Dept. of Computer Science, University of New Mexico, Albuquerque, NM, USA.
One hypothesis for the onset of Alzheimer's disease associates the aggregation of excess tau-protein on microtubules with the hindrance of cargo transport by molecular motors. This hypothesis has motivated experimental and modeling studies of kinesin procession in the presence of such obstacles. Very recently, it has been shown that kinesin's neck linker length is closely related to its ability to bypass obstacles and avoid early detachment from the microtubule, by sidestepping to adjacent microtubule tracks. Here we present results from kinetic models that explicitly account for such sidestepping by analyzing published experimental single-molecule data on the processivity of kinesin-1 and kinesin-2, both with and without obstacles. The mechanochemistry is